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Chiral derivatives of xanthones: investigation of enantioselectivity as inhibitors of cyclooxygenases (COX-1 and COX-2) and binding interaction with human serum albumin

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U.PORTO FACULDADE DE FARMÁCIA UNIVERSIDADE DO PORTO

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Chiral derivatives of xanthones: investigation of enantioselectivity as inhibitors of cyclooxygenases (COX-1 and COX-2) and binding interaction with human serum albumin

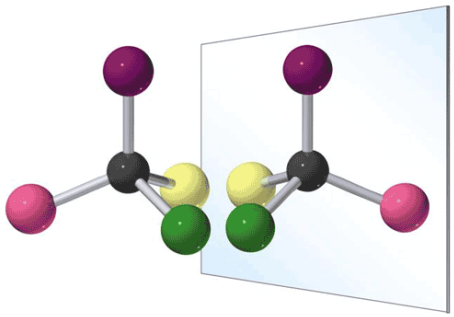
Graphical Abstract

Abstract:
Searching for new enantiomerically pure chiral derivatives of xanthones (CDXs) with potential pharmacological properties has remained an area of interest of our group, namely those with anti-inflammatory activity. Herein, we describe *in silico* studies and *in vitro* inhibitory assays of different enantiomeric pairs of CDXs. The evaluation of the inhibition of cyclooxygenases (COX-1 and COX-2) activities was performed by using the COX Inhibitor Screening Assay Kit. Docking simulations between the small molecules (CDXs, known ligands and decoys) and the enzyme targets were undertaken with AutoDock Vina embedded in PyRx – Virtual Screening Tool software. All the CDXs evaluated exhibited COX-1 and COX-2 inhibition potential as predicted.
Considering that the (*S*)-(-)-enantiomer of the nonsteroidal anti-inflammatory drug Ketoprofen preferentially binds to albumin, resulting in lower free plasma concentration than (*R*)-(+)-enantiomer, protein binding affinity for CDXs was also evaluated by spectrofluorimetry. For some CDXs enantioselectivity was observed.

Keywords: chiral derivatives of xanthones, cyclooxygenase, albumin, enantioselectivity

Introduction

CHIRALITY IN DRUG DEVELOPMENT



Chirality plays a key role in biochemical events

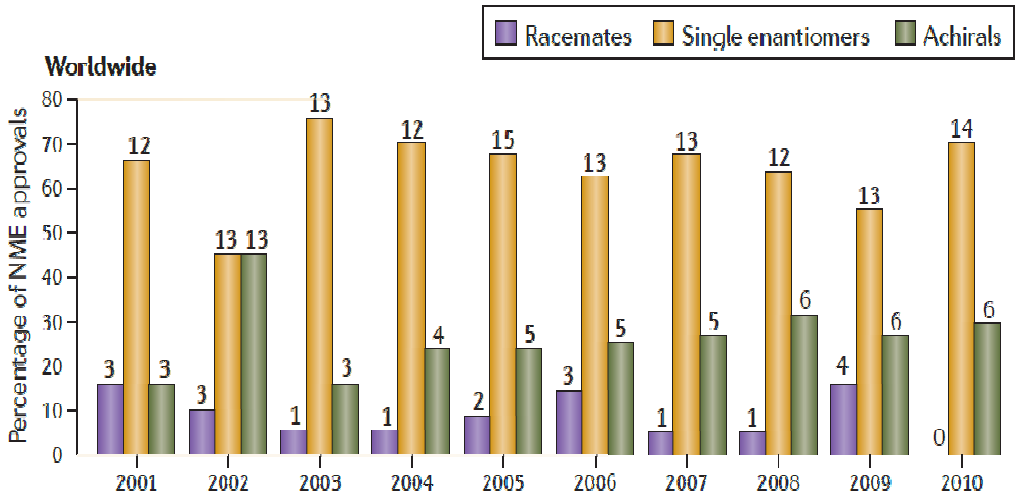
Chiral molecular recognition strongly influences drug action and efficacy

Frequently only one of the two enantiomers exerts the desired effect
Eutomer/Distomer

Major importance in drug discovery and design

***De novo* design**

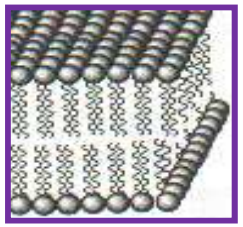
Chiral switches



Agranat, S. R. Wainschtein, and E. Z. Zusman, *Nat. Rev. Drug Discov.*, 2012, 11, 972–973.

Introduction

INFLAMMATORY PATHWAY AND ITS MEDIATORS



Membrane phospholipids

Arachidonic acid cascade

COX: cyclooxygenase

Diverse physical, chemical, inflammatory and mitogenic stimuli

Phospholipase A_2

Arachidonic acid

COX

HOX

Cyclooxygenase-1

Prostaglandin G_2

Prostaglandin H_2

Prostaglandin G_2

Prostaglandin H_2

Cyclooxygenase-2

Tissue-specific isomerases

Prostacyclin

Thromboxane A_2

Prostaglandin D_2

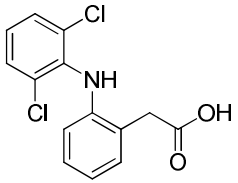
Prostaglandin E_2

Prostaglandin $F_{2\alpha}$

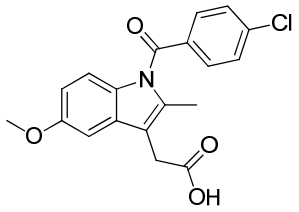
FitzGerald, G. A., *Nat Rev Drug Discov*, 2003, 2(11), 879-890.

ENANTIOSELECTIVITY IN INFLAMMATORY ACTIVITY

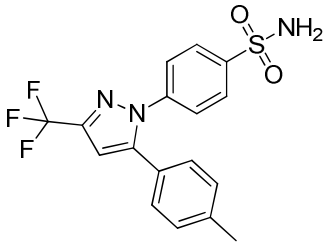
Examples of nonsteroidal anti-inflammatory drugs (NSAIDs):



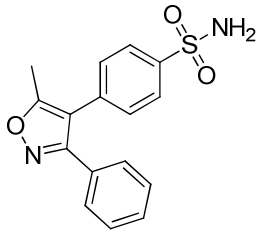
Diclofenac



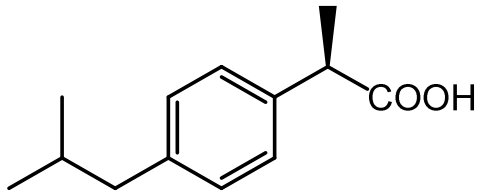
Indomethacin



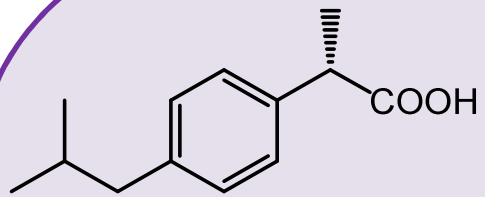
Celecoxib



Valecoxib



(*R*)-(-)-Ibuprofen



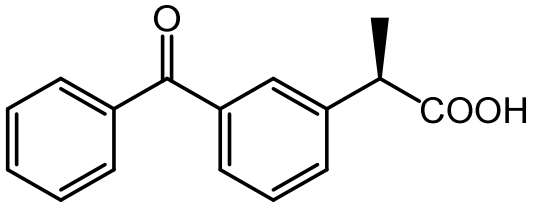
(*S*)-(+)-Ibuprofen

Eutomer

Introduction

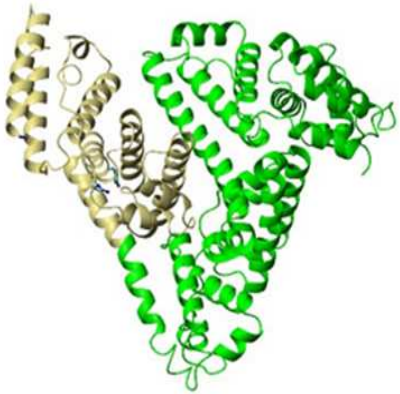
Enantioselectivity in Albumin binding

NSAID

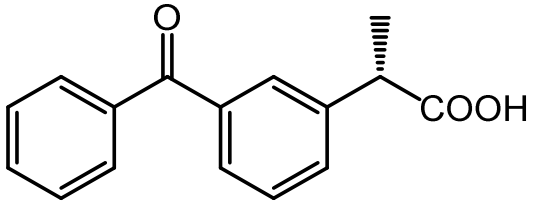


(*R*)-(+)- Ketoprofen

Higher affinity



Human Serum Albumin (HSA)



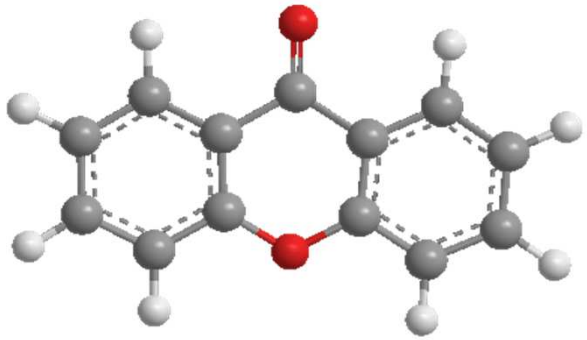
(*S*)-(-)- Ketoprofen

higher free plasma concentration

Evans, S. E. *et al. Trends Env Anal Chem*, 2014, 1(0), e34-e51.

Introduction

XANTHONE – A PRIVILEGED STRUCTURE



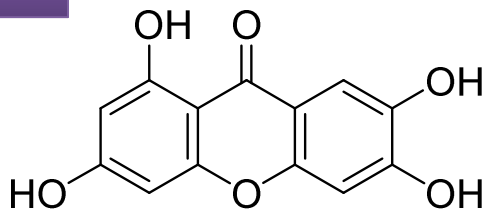
Capable of binding to multiple targets, and consequently, with appropriate structure modifications, could exhibit multiple activities

Different substituents
Natural and synthetic origin

Wide range of biological activities

- **Anti-hypertensive**
- **Anti-inflammatory**
- **Antibacterial**
- **Anti-epileptic**
- **Anti-arrhythmic**
- **Antitumor (…)**

Example



Norathyriol

Pinto, M., E. Sousa, *et al.* Curr Med Chem, 2005, 12(21), 2517-2538.
Shagufta and I. Ahmad, *Eur J Med Chem*, 2016, 116, 267-280.

Introduction

CHIRAL DERIVATIVES OF XANTHONES

New chiral derivatives of xanthones: Synthesis and investigation of enantioselectivity as inhibitors of growth of human tumor cell lines

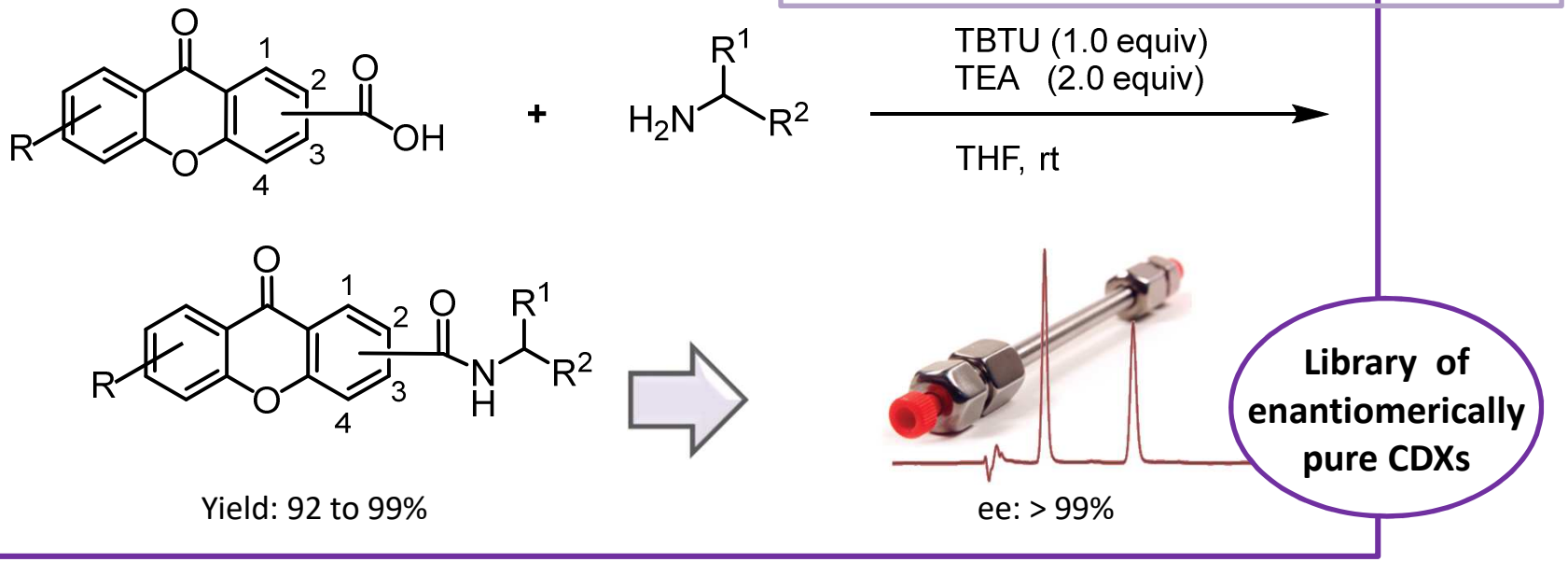
Bioorganic & Medicinal Chemistry 22 (2014) 1049–1062

Carla Fernandes[a,b,c,†], Kamonporn Masawang[a,d,†], Maria Elizabeth Tiritan[a,c,e], Emília Sousa[a,b,c], Virgínia de Lima[f], Carlos Afonso[a,b,c], Hassan Bousbaa[a,c,e], Wanwisa Sudprasert[d], Madalena Pedro[a,e,*], Madalena M. Pinto[a,b,c,*]

Synthesis of new chiral xanthone derivatives acting as nerve conduction blockers in the rat sciatic nerve

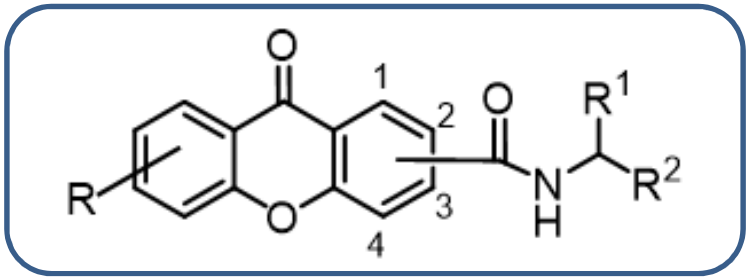
Carla Fernandes[a,b,1], Laura Oliveira[c,1], Maria Elizabeth Tiritan[b,d], Luís Leitao[c], Angelo Pozzi[c], José Bernardo Noronha-Matos[c], Paulo Correia-de-Sá[c,*], Madalena M. Pinto[a,b,**]

European Journal of Medicinal Chemistry 55 (2012) 1–11



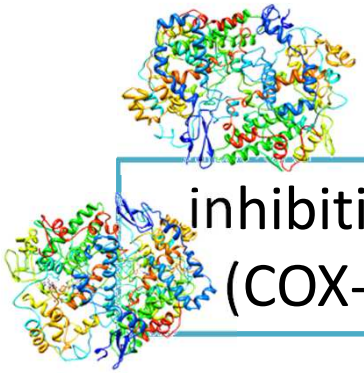
CDX: Chiral derivative of xanthone;TBTU: *O*-(Benzotriazol-1-yl)-*N*-*N*-*N'*-*N'*-tetramethyluronium tetrafluoroborate; TEA: Triethylamine; THF: Tetrahydrofuran; ee : enantiomeric excess.

Aims



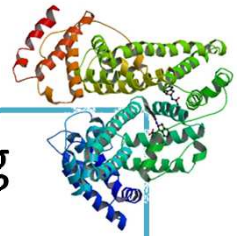
enantiomeric pairs of CDXs

in silico studies and *in vitro* assays



inhibition of cyclooxygenases (COX-1 and COX-2) activity

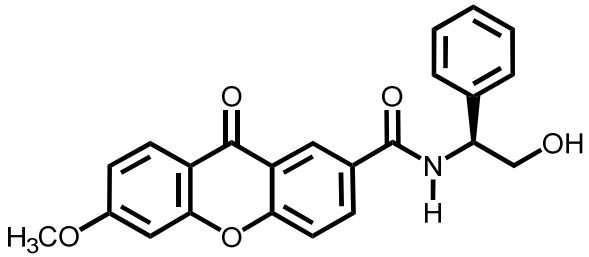
protein binding affinity

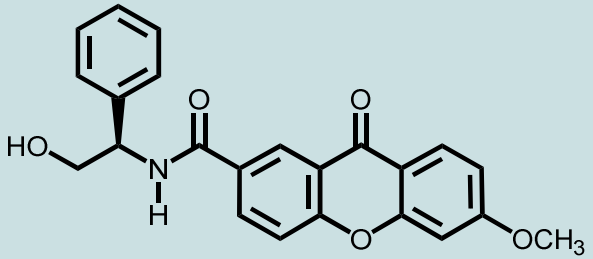


Results and discussion

ENANTIOMERIC PAIRS OF CDXS

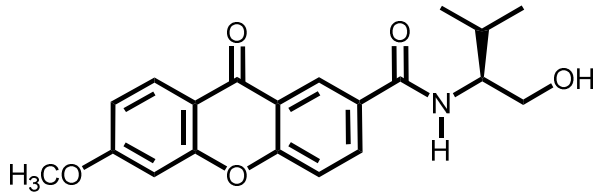
(*S*)-XEGOL2

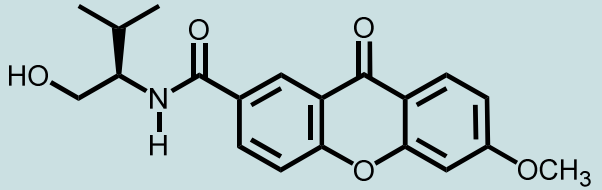




(*R*)-XEGOL2

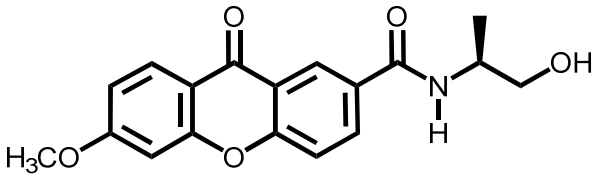
(*S*)-XEVOL

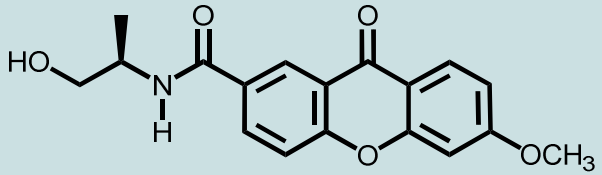




(*R*)-XEVOL

(*S*)-X2AP1



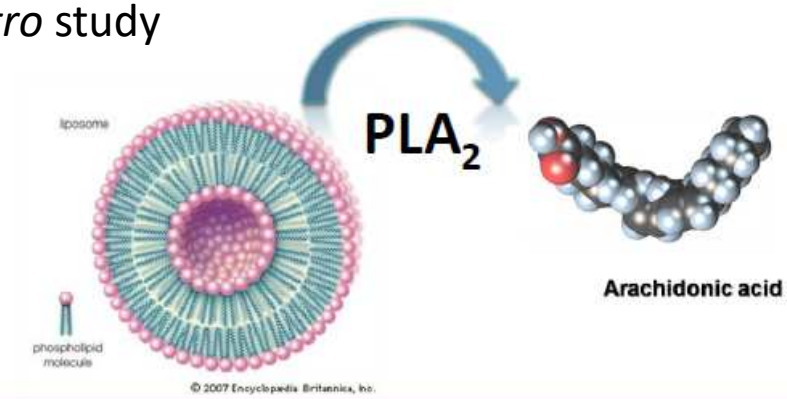


(*R*)-X2AP1

Results and discussion

INHIBITION OF CYCLOOXYGENASES

In vitro study

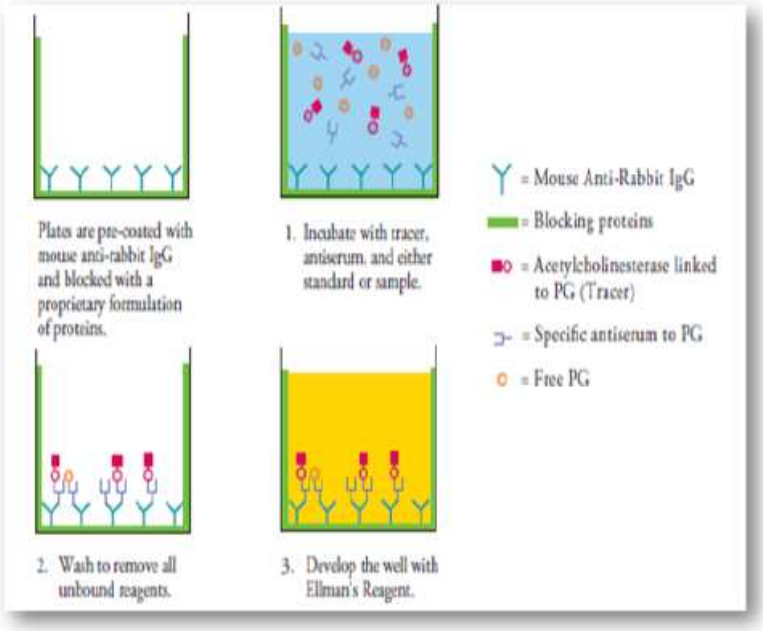


COX-1
COX-2 → PGH_2

LUVs → ADIFAB → PLA2+DRUG

ADIFAB: AcryloDated Intestinal fatty acid binding protein

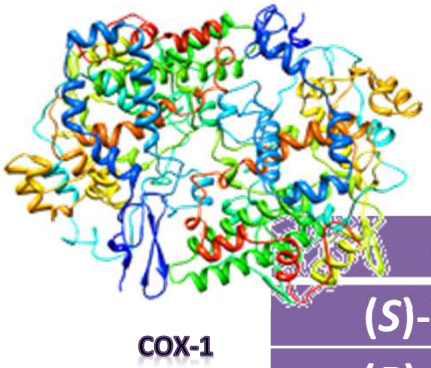
The presence of an inhibitor leads to a decrease in the release of fatty acid which generates a minor decrease of the fluorescence of ADIFAB (λ_{em}=432 nm). This allows the calculation of the relative inhibition (%).



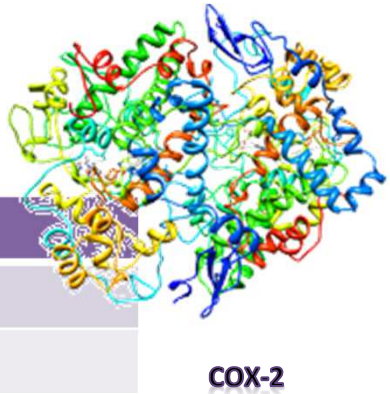
Gelb, H., Jain, M. K., Berg, O., *Bioorg. Med. Chem. Lett.*, 1992, 1335.
Dixon D.A., *et al.*, *J Biol Chem*, 2000, 275, 11750–11757.

Results and discussion

INHIBITION OF CYCLOOXYGENASES



COX-1 and COX-2 inhibitory effects of CDXs



CDX	COX-1	COX-2
(*S*)-XEGOL2	87.6 ± 2.1	80.1 ± 12.8
(*R*)-XEGOL2	79.6 ± 5.0	84.7 ± 5.7
(*S*)-XEVOL	82.9 ± 5.2	85.7 ± 4.5
(*R*)-XEVOL	66.8 ± 1.6	73.2 ± 0.4
(*R*)-X2A1P	75.2 ± 9.0	75.1 ± 7.2
(*S*)-X2A1P	91.7 ± 10.7	93.4 ± 11.4
Indomethacin	83.2 ± 6.4	80.7 ± 9.5

Results are given as % of inhibition and are expressed as mean ± standard deviation of two independent experiments.

The concentration of CDXs was 20 µmol.L^{-1}.
Indomethacin 1 µmol.L^{-1} was used as positive control.

Results and discussion

ALBUMIN BINDING

In vitro study

Solutions of increasing concentrations of CXD (Phosphate buffer, pH 7.4, *I*=0,1 M)

Adding HSA (final concentration 2 µM)

HSA + CDX

Fluorimetric analysis
λ_{exc} = 280 nm, λ_{em} = 330 nm

$$\%QUENCHING = \frac{y_{max}}{1 + \frac{K_d}{[ligand]}}$$

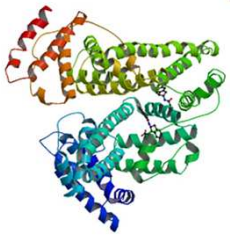
Langmuir model

$$\%QUENCHING = \frac{y_{max}^{n}}{1 + \frac{K_d}{[ligand]}}$$

Number of the binding sites (*n*)

$$\Delta G = -RT\ln K$$

$$HSA + ligand \underset{K_d}{\overset{K_b}{\rightleftharpoons}} HSA\text{-}ligand$$



Results of the binding parameters

CDX	K_d (µM)	Y_{max}	n	ΔG binding (Kcal/mol) 25 ^{0}C
(*S*)-XEGOL2	23.6 ± 0.8	105.3 ± 0.4	1	-1.9 ± 0.1
(*R*)-XEGOL2	61.8 ± 6.5	109.6 ± 1.6	1	-2.4 ± 0.2
(*S*)-XEVOL	24.7 ± 1.1	107.4 ± 5.4	1	-1.9 ± 0.1
(*R*)-XEVOL	29.2 ± 0.9	108.2 ± 0.2	1	-2.0 ± 0.1
(*R*)-X2A1P	26.4 ± 1.2	113.2 ± 1.4	1	-1.9 ± 0.1
(*S*)-X2A1P	31.4 ± 2.0	116.2 ± 0.6	1	-2.0 ± 0.2

K_d (µM) < 100 µM

Results and discussion

DOCKING STUDIES

Ligands

CDXs and positive controls

Drawn and minimized

Decoys and known ligands

From DUD - *a directory of useful decoys*

Negative controls

NCI compound database - based on structural parameters of CDXs

Targets

Protein Data Bank of Brookhaven

ovine COX-1 (PDB code: 3n8x)
murine COX-2 (PDB code: 1cx2)
human albumin (PDB code: 2bxg)

⬇

Water molecules and crystal ligands removed

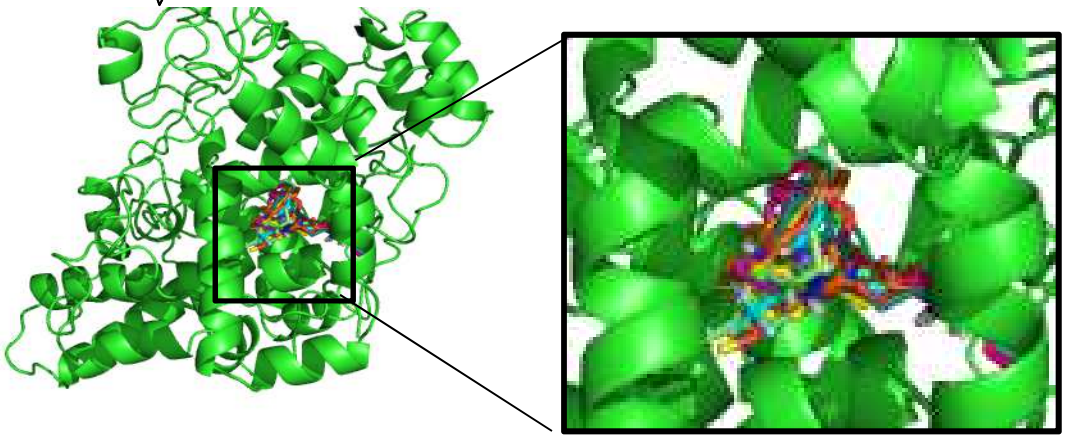
Flexible and adaptable | **AutoDock Vina** | **Rigid units**

Lowest binding energy docking poses of each compound were chosen

Results and discussion

DOCKING STUDIES – COX-1

- ✓ **Positive controls**
- ✓ **Known ligands**
- ✓ **Decoys**
- ✓ **CDXs**

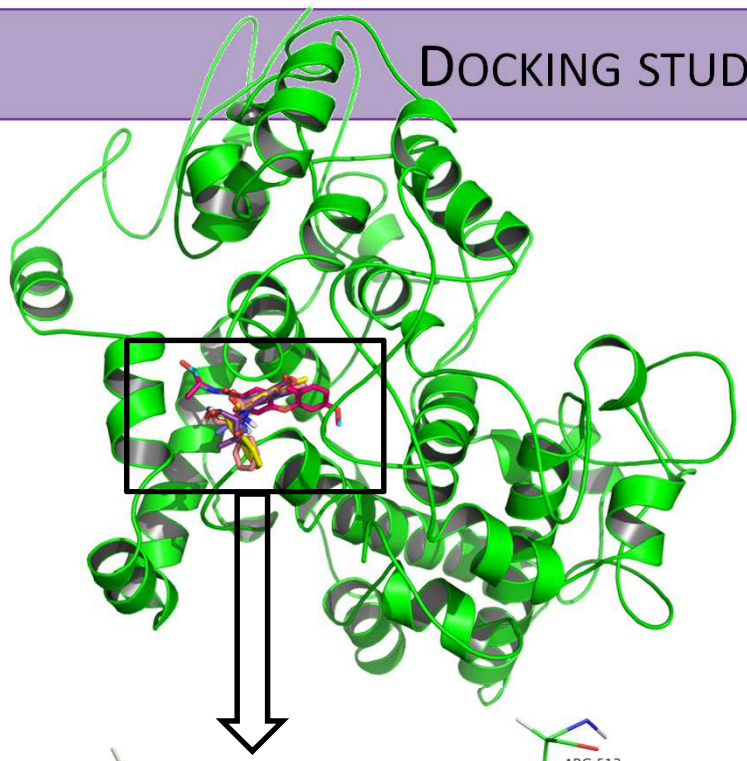


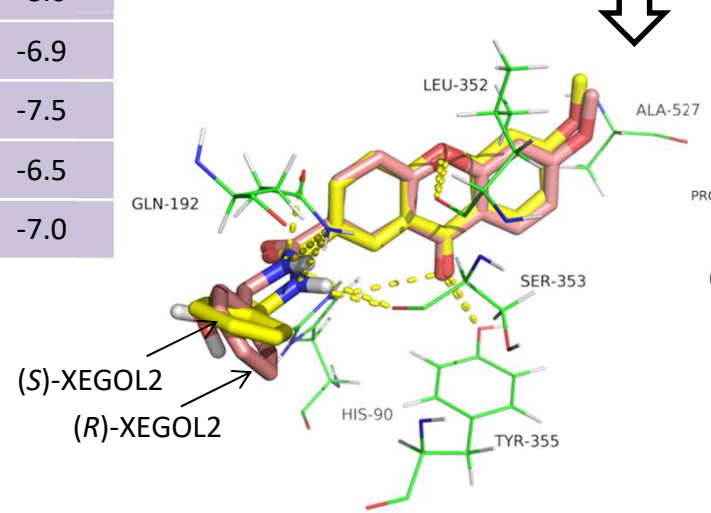
COX-1 binding energy (Kcal/mol)		
Known ligands	Diclofenac	-6.1
	Indomethacin	-5.1
	Naproxen	-7.8
	Piroxicam	-5.2
Ligands from database		-7.8
Decoys from database		-7.3
(*R*)-XEGOL2		-4.2
(*S*)-XEGOL2		-4.5
(*R*)-X2A1P		-5.3
(*S*)-X2A1P		-5.6
(*R*)-XEVOL		-3.4
(*S*)-XEVOL		-5.4

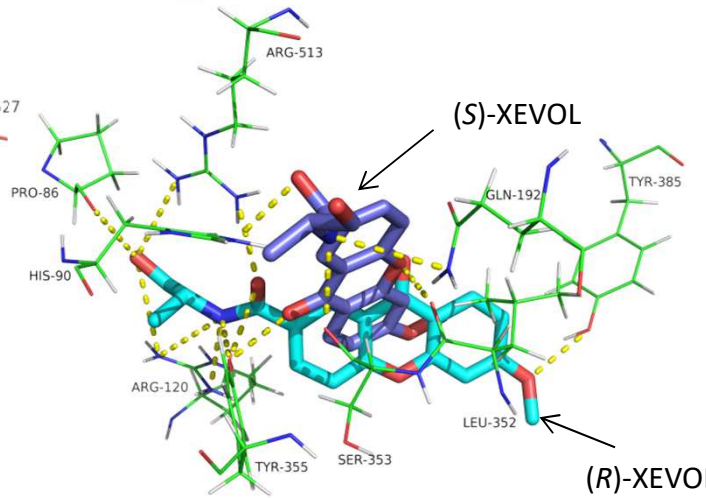
Results and discussion

DOCKING STUDIES – COX-2

COX-2 binding energy (Kcal/mol)		
Known ligands examples	Diclofenac	-7.9
	Indomethacin	-7.9
	Celecoxib	-11.5
	Valecoxib	-9.5
Ligands from database		-9.3
Decoys from database		-7.6
(*R*)-XEGOL2		-7.8
(*S*)-XEGOL2		-8.0
(*R*)-X2A1P		-6.9
(*S*)-X2A1P		-7.5
(*R*)-XEVOL		-6.5
(*S*)-XEVOL		-7.0







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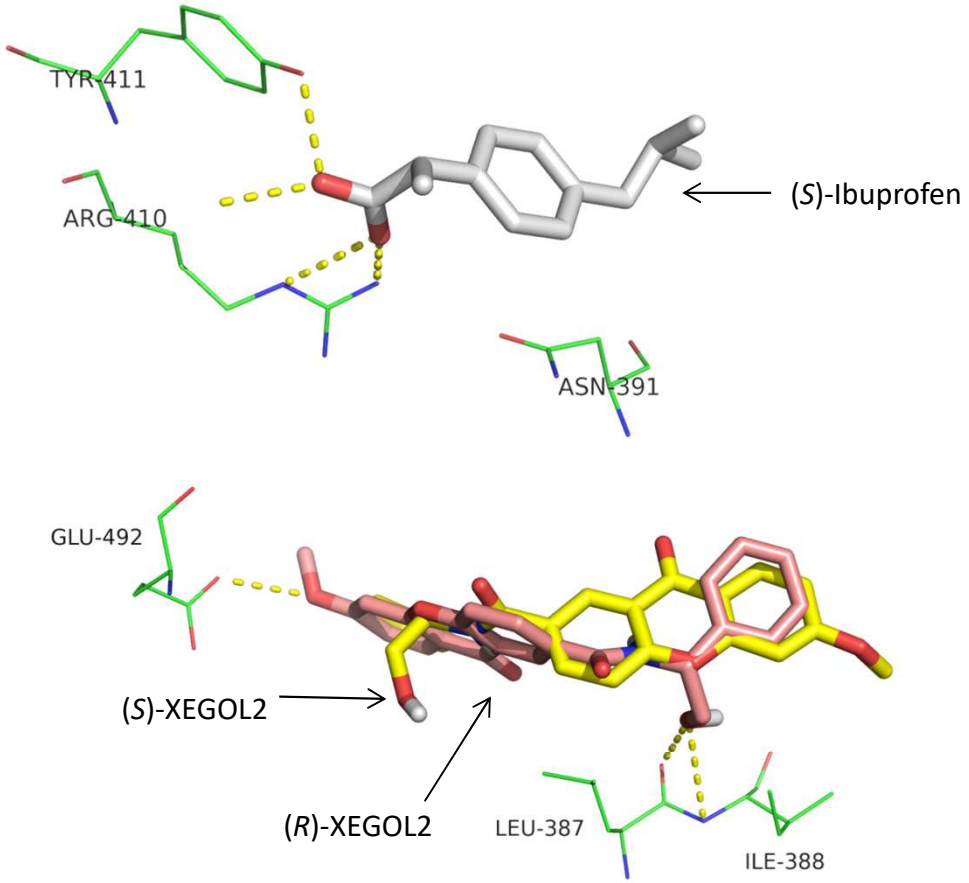
Results and discussion

DOCKING STUDIES – ALBUMIN

Albumin binding energy (Kcal/mol)		
Known ligands	Azaprozone	-5.9
	Diazepam	-7.1
	Fusidic acid	-5.8
	Ibuprofen	-7.3
	Iophenoxid acid	-4.4
	Naproxen	-7.9
	Warfin	-8.5
(*R*)-XEGOL2		-7.3
(*S*)-XEGOL2		-7.0
(*R*)-X2A1P		-7.2
(*S*)-X2A1P		-7.0
(*R*)-XEVOL		-7.2
(*S*)-XEVOL		-7.2



Conclusions

Considering the inhibition of cyclooxygenases (COX-1 and COX-2):

- all the CDXs evaluated exhibited COX-1 and COX-2 inhibition potential in *in vitro* assays,
- the inhibitory effects were very similar for the same enantiomeric pair as well as for both COXs,
- no significative difference was found between known ligands and decoys docking scores on COX-1; therefore, no reliable conclusions can be taken from test ligands binding affinity to COX-1,
- XEGOL2 enantiomeric pair is predicted to show more affinity towards COX-2, presenting docking scores similar to known ligands, such as diclofenac and indomethacin.

Considering the HSA binding affinity:

- all CDXs demonstrated to bind with high affinity to HSA potential in *in vitro* assays,
- XEGOL2 enantiomeric pair exhibited enantioselectivity,
- *in silico* studies CDXs confimed that they bind to albumin serum protein, as they have docking scores similar to positive controls such as ibuprofen and diazepam.

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